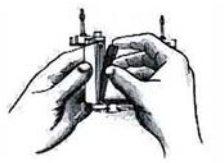
Instruction Manual for IDGel™ from DGel Electrosystem Inc

For complete protocol and product information, visit www.idgel.com

Running IDGel™



- Using IDGel™ in a Bio-Rad Gel Running Apparatus

  Prior to inserting the gel in the apparatus
  - Remove the gasket from the inner frame, then turn the gasket around so that the flat side is facing outwards and re-insert into the inner frame
- Using IDGel™ in an Invitrogen Gel Running Apparatus
  - Remove gel from packet and insert into the gel running apparatus.
  - Along the gel cassette, slide the plastic insert enclosed in the IDGel™ box.
  - Close the chamber clamp according the manufacturer's instructions

Loading and running IDGel™ Precast Gels:

- Dilute the samples to be analyzed with the appropriate volume of sample buffer in sealable micro centrifuge tubes.
- For protein electrophoresis, heat the samples for 5 min at 100°C, if denaturing conditions are used (SDS-PAGE)
- Load the samples.
- Run the gel(s) at an appropriate power supply setting (see table below*)

Buffer system	Tris-glycine SDS (Laemmli)		Tris-glycine (native)	Tris-Tricine SDS	Tris-acetate EDTA
Voltage	**150 VDC**	**250 VDC**	**125 VDC**	**125 VDC**	**200 VDC**
Approx per gel: START	30-40 mA	55-65 mA	20-30 mA	45--55 mA	45-55 mA
Approx per gel: END	15-25 mA	30-40 mA	10-20 mA	20-30 mA	20-30 mA
Current/Wattage: START	3-7 W	12-16 W	2-6 W	4-8 W	8-12 W
Current/wattage: END	2-6 W	10-14 W	1-5 W	2-6 W	3-7 W
Time	60 min	35 min	100 min	60 min	60 min

Attention:

"IDGel™ is delivered without combs, ready-to-use. Note that, due to the absence of a comb, occasionally the well bottom may seem slightly curved or not flat. **This is due to cold temperature during packaging and this normal phenomenon will not affect the gel nor band migration.** In fact, the wells will return to their normal flat position when the temperature increases or when buffer is added. Use the gels immediately when removed from the pouch."

Opening the IDgel™ cassette

- Hold the cassette with both hands with the notched plate facing you. Press both thumbs onto the larger plate and at the same time pull both spacer extensions with another finger in the opposite direction.
- Completely separate the cassette halves by slowly pulling them apart with your fingers. Once the gel is free from one of the cassette halves, invert it carefully over the staining, fixing or transfer solution. Using a thin spatula, or one of the spacers, separate the gel from the cassette wall starting at one corner.

In general

Maximum voltage 250 VDC. The plastic is recyclable (recycling code 1). The acrylamide matrix is colored to see the wells easily. The color will migrate with the migration front. It is non-toxic and does not interfere with proteins or nucleic acids, or with further analysis of your material (Zymogram, Western blot, etc...)

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Guide to protein staining procedures

Coomassie Blue R-250 staining of proteins.

Staining solution	Ingredient	Amount	Final Stock Conc.
	Brilliant Blue R-250*	1 g	0.1% (w/v)
	Methanol	500 ml	50% (v/v)
	Glacial Acetic Acid	100 ml	10% (v/v)
	Distilled water	To 1 liter	.
Destaining solution	**Ingredient**	**Amount**	**Final Stock Conc.**
	Methanol	250 ml	5% (v/v)
	Glacial Acetic Acid	80 ml	8% (v/v)
	distilled water	to 1 liter	

*completely dissolve the dye in methanol and then add the other components

Protocol:

- Place the gel constant shaking in 100 ml of Staining solution for 20 min
- Wash the gel with 100 ml distilled water for 10 seconds
- Destain the gel under constant shaking in 200 ml of Destaining solution with shaking for 30 min
- Repeat step 3 with fresh Destaining solution until the background is clear (generally, 3 X 30 min; however, time will vary with gel conc.)

Silver staining of proteins

Place the gel in distilled water **Gel fixing solution1**	**Ingredient**	**Amount**	**Final Stock Conc.**
	Ethanol	50 ml	50%
	Glacial Acetic Acid	10 ml	10% (v/v)
	distilled water	to 100 ml	
Gel fixing solution2	Ingredient	Amount	Final Stock Conc.
	Ethanol	90 ml	30% (v/v)
	distilled water	to 300 ml	
Sensitizer	Ingredient	Amount	Final Stock Conc.
	200 mM Na thiosulfate	400 microliters	0.8 mM
	distilled water	to 100 ml	
Staining solution*	Ingredient	Amount	Final Stock Conc.
	Silver nitrate	0.2 g	11.8 mM
	37% formaldehyde	54 microliters	0.02% (v/v)
	distilled water	to 100 ml	
	*formaldehyde should be added just before use		
Developing solution*	Ingredient	Amount	Final Stock Conc.
	Na carbonate	6.0 g	566 mM
	200 mM Na thiosulfate	10 microliters	0.02 mM
	37% formaldehyde	54 microliters	0.02% (v/v)
	*formaldehyde should be added just before use		
Stop solution	Ingredient	Amount	Final Stock Conc.
	Glacial Acetic Acid	5 ml	5% (v/v)
	distilled water	to 100 ml	

Protocol

Note: All steps of the following protocol should be performed under constant gentle shaking. A sufficient amount of solutions should be used; typically, 100 ml is suggested in each step to cover the gel.

1. Fix in Gel Fixing solution 1 for 30 min
2. Wash three times in Gel Fixing solution 2 (each time for 10 min)
3. Treat with Sensitizer for 1 min
4. Rinse twice with distilled water for 1 min each time
5. Stain with Staining solution for 20 min
6. Rinse twice with distilled water for 1 min each time
7. Develop with Developing solution until the protein bands are readily visible (generally 5 to 10 min; avoid prolonged times as this will just increase the background staining)
8. Decant and replace with Stop solution for 10 min
9. Store in distilled water.

WESTERN BLOTTING

Note: The following protocols for Western blotting provide general information and conditions that may be applied to most transfer procedures. However, careful optimization should be considered if quantitative recovery of a particular protein is required since blotting efficiency is dependent on buffer composition, blotting time and current, membrane used, etc.

Transfer with a tank blotting system

Transfer buffer (cool to 4C before use)

Ingredient	Amount	Final Stock Conc.
Tris-base	3.0 g	25 mM
Glycine	14.4 g	192 mM
Methanol*	100 - 200 ml	10 - 20 %
distilled water	to 1 liter	

*10% methanol is recommended when using PVDF blotting membranes while

20% is recommended when using nitrocellulose-blotting membranes.

Transfer protocol

1. Pre-soak the porous pads and two pieces of filter paper cut to the size of the gel (ex. Whatman 3MM paper) in transfer buffer for 5 min
2. Cut a piece of blotting membrane to the size of the gel and equilibrate in transfer buffer. If a PVDF membrane is used, rinse in methanol for 2 min before equilibration in the transfer buffer for 5 min
3. Remove the gel from the cassette and equilibrate in transfer buffer for 5 min
4. Carefully assemble the transfer stack. Remove all air bubbles between the layers. If you do NOT do this then the air bubbles will affect the efficiency of the transfer process and may leave blank spots on the blotting membrane
5. Place the stack into the tank unit making sure it is in the PROPER ORIENTATION. Fill the chamber with transfer buffer
6. Transfer at room temperature at 100 mA for 60 to 120 min
7. When the transfer is complete, turn off the power and remove the layers until you reach the blotting membrane
8. Remove the blotting membrane with a pair of forceps, rinse in distilled water and air-dry for 1 min.

Transfer with a semi-dry system

Transfer buffer (cool to 4C before use)

Ingredient	Amount	Final Stock Conc.
Tris-base	0.3 g	25 mM
Glycine	1.44 g	192 mM
Methanol	10.0 ml	10% (v/v)
distilled water	to 100 ml	

Transfer protocol (wear gloves throughout the procedure)

1. Pre-soak four pieces of blotting membrane to the size of the gel and equilibrate in transfer buffer. If a PVDF membrane is used, rinse it in methanol for 2 min before equilibration in the transfer buffer for 5 min
2. Remove the gel from the cassette. Carefully assemble the transfer stack on the anode (+). Remove all air bubbles between the layers. Air bubbles will affect the efficiency of the transfer process and may leave blank spots on the blotting membrane
3. Transfer at room temperature at 0.8 mA/cm^2 of gel (limit voltage to 15 V) for 60 min
4. When the transfer is complete turn off the power and remove layers until you reach the blotting membrane
5. Remove the blotting membrane with a pair of forceps, rinse in distilled water and air-dry for 1 min

Monitoring of protein transfer

The efficiency of transfer can be monitored using prestained protein markers. Alternatively, the extent of protein transfer can be determined by staining the polyacrylamide gel after the transfer is complete or by staining the proteins directly on the blotting membrane. Proteins on solid support can be stained with dyes such as India ink, Amino Black or Coomassie Blue. A recommended stain is Ponceau S, which is reversible. The detection limit is 1 to 2 micrograms.

Ponceau S staining for proteins on solid support

Ingredient	Amount	Final Stock Conc.
Ponceau S	0.2 g	0.2% (w/v)
Glacial Acetic Acid	1 ml	1% (v/v)
distilled water	to 100 ml	

Protocol

1. If the blotting membrane is dry, re hydrate it with water (or methanol, if PVDF) for 5 min
2. Stain the blotting membrane under constant shaking with the Ponceau S solution for 5 min
3. Destain the membrane under constant shaking with distilled water
4. If required, wash the blot with 0.1N NaOH to remove the stain completely